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## ORIGINAL ARTICLE

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# CIRCADIAN DEPENDENCE OF MANUAL THROMBUS ASPIRATION EFFICACY IN PATIENTS WITH ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION UNDERGOING PRIMARY PERCUTANEOUS CORONARY INTERVENTION

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## ABSTRACT

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### INTRODUCTION

The clinical benefits of routine intracoronary thrombus aspiration during primary percutaneous coronary intervention (PCI) in patients with ST-segment elevation myocardial infarction (STEMI) remain uncertain. Previous studies have suggested a circadian influence of time-of-day symptom onset on myocardial infarct size and effectiveness of reperfusion strategies in patients with STEMI. We aimed to assess the impact of circadian rhythms on the clinical efficacy of manual thrombus aspiration for patients with STEMI undergoing primary PCI.

### METHODS

We conducted an observational cohort study of consecutive patients enrolled in the large, prospective, multicenter Acute Myocardial Infarction in Switzerland (AMIS) Plus registry and treated with primary PCI for STEMI within 12 hours of symptom onset. STEMI patients undergoing primary PCI with (thrombus aspiration group) or without (PCI-alone group) manual thrombectomy were divided in four time groups based on the time-of-day symptom onset: group 1 (00:00-05:59), group 2 (06:00-11:59), group 3 (12:00-17:59) and group 4 (18:00-23:59). Propensity-score matching analysis was performed to account for confounders and potential bias between the treatment groups. The primary outcome was in-hospital all-cause mortality. The secondary endpoint was myocardial infarct size, measured by peak creatine kinase (CK) release. Periodic sinusoidal regression analysis was used to determine circadian dependence of time-of-day symptom onset on the primary outcome.

### RESULTS

Between January 2009 and March 2014, 3'648 patients with STEMI were treated with primary PCI within 12 hours of symptom onset, of whom 1'800 (49%) underwent manual thrombus aspiration. After propensity-score matching, a cohort of 2'860 patients (1'430 patients in thrombus aspiration and PCI-alone groups) was included in the primary analysis. In-hospital all-cause mortality occurred in 4.3% in the thrombus aspiration group and 3.4 % in the PCI-alone group (hazard ratio 1.28; 95% confidence interval 0.87-1.89; p=0.20). A significant influence of time-of-day symptom onset on manual thrombus aspiration efficacy with respect to myocardial salvage was demonstrated in patients undergoing manual thrombectomy. Largest final myocardial infarct size was observed when symptom onset occurred

between the 18:00-05:59 interval (peak CK levels, group 1, 2'723±148 U/l; group 2, 2'493±105 U/l; group 3, 2'550±106 U/l, and group 4, 2'952±144 U/l; p=0.044), whereas no significant difference was demonstrated in patients treated with PCI only. After periodic sinusoidal regression analysis, a significant circadian relationship between time-of-day symptom onset and final myocardial infarct size was demonstrated in patients undergoing manual thrombus aspiration (p<0.001).

## **CONCLUSION**

In our large, real-world registry of unselected patients undergoing primary PCI for STEMI, routine manual thrombectomy as compared with PCI alone did not reduce the risk of in-hospital all-cause death. We observed a circadian dependence of manual TA efficacy with greatest myocardial salvage in patients with symptom onset between 06:00 and 17:59.

## INTRODUCTION

Primary percutaneous coronary intervention (PPCI) is the most effective reperfusion strategy for patients with acute ST-segment elevation myocardial infarction (STEMI) (1). Distal embolization of thrombus resulting in microvascular obstruction and failure to restore optimal microvascular perfusion remains a major limitation of PPCI and has been associated with increased risk of mortality (2, 3). Manual thrombus aspiration (TA) during PPCI has been introduced as an adjunctive strategy with the intent to reduce thrombus burden, prevent distal embolization, increase microvascular perfusion and improve outcomes. The clinical benefit of routine manual intracoronary TA during PPCI remains uncertain owing to the conflicting results reported by randomized clinical trials. The *Thrombus Aspiration during Percutaneous Coronary Intervention in Acute Myocardial Infarction Study* (TAPAS) single-center randomized trial involving 1'071 patients, which was not designed or powered for clinical outcomes, suggested not only improved myocardial reperfusion but a nearly 50% reduction in 1-year mortality among STEMI patients undergoing manual TA, as compared to PPCI alone (4) (5). Contrariwise, two recent large-scale multicenter randomized trials powered for hard clinical endpoints, the *Thrombus Aspiration During ST-Segment Elevation Myocardial Infarction* (TASTE) trial including 7'244 patients (6, 7) and the *Trial of Routine Aspiration Thrombectomy with PCI versus PCI Alone in Patients with STEMI* (TOTAL) study involving 10'732 patients (8), and subsequent meta-analyses (9, 10) have consistently demonstrated that aspiration thrombectomy did not significantly reduce the risk of all-cause mortality and adverse clinical outcomes, when compared with PCI alone. Accordingly, both the European Society of Cardiology and American College of Cardiology/American Heart Association guidelines do not recommend the routine use of aspiration thrombectomy during PPCI for STEMI, while selective or bailout manual TA may be considered to improve Thrombolysis In Myocardial Infarction (TIMI) 3 flow or prevent stent thrombosis (11, 12). However, the optimal selection of STEMI patients who might derive clinical benefit from manual TA aspiration during PPCI remains unclear.

Circadian rhythms have been shown to exert a critical influence on the pathogenesis of acute myocardial infarction (MI) (13). Multiple recent large-scale registry-based studies have demonstrated a time-of-day-dependent variation of MI size among patients with STEMI undergoing PPCI with larger infarct size and increased in-hospital mortality rates around midnight, regardless of the total ischemic time (14-19). Furthermore, previous studies have confirmed a substantial circadian variation in the effectiveness of reperfusion strategies for patients with STEMI, such as fibrinolysis and PPCI, with lowest reperfusion rates and worse clinical outcomes during the early morning hours (20-22). These data suggest a circadian dependence of myocardial tolerance to the ischemia/reperfusion (I/R) injury during STEMI.

We aimed to assess the impact of circadian rhythms on the clinical benefit of manual intracoronary TA in a large real-world cohort of STEMI patients undergoing PPCI in contemporary clinical practice. We hypothesized that the time-of-day onset at which the myocardium is subjected to I/R injury may influence the effectiveness of manual intracoronary thrombectomy during PPCI.

## **METHODS**

We conducted a retrospective analysis of patients included in AMIS (*Acute Myocardial Infarction in Switzerland*) Plus, a large, ongoing, nationwide, multicenter registry enrolling consecutive patients admitted with acute coronary syndrome (ACS) to hospitals in Switzerland, to evaluate the circadian relationship between time-of-day symptom onset and intracoronary thrombectomy effect during PPCI for STEMI.

### **Study database**

AMIS Plus was founded in 1997 with the goal to understand the transfer, use and practicability of knowledge gained from randomized trials, and to generate data for the planning of subsequent prospective randomized studies. From 106 hospitals treating ACS in Switzerland, 82 hospitals temporarily or continuously enrolled patients and AMIS Plus currently includes data from more than 50'000 patients with ACS. The design of the registry has been described previously (23). AMIS Plus collects prospectively on a computer-based database 230 items including medical history, comorbidities, cardiovascular risk factors, clinical presentation, out-of-hospital management, early in-hospital management, reperfusion therapy, hospital course, diagnostic tests used or planned, length of stay, discharge medication and discharge destination, immediate drug treatment and discharge medication. Participating centers provide blinded data for each patient through standardized internet-based or paper-based questionnaires and are strongly encouraged to enroll all patients fulfilling the inclusion criteria to avoid selection bias. Hospital data are provided and completed by the treating physician or a trained study nurse. All data are checked for completeness, plausibility and consistency by the AMIS Plus Data Center in the Institute of Social and Preventive Medicine at the University of Zurich and treating physicians or study nurses are queried when necessary. The AMIS Plus project is officially supported by the Swiss Societies of Cardiology, Internal Medicine and Intensive Care Medicine.

Baseline characteristics, treatment strategies and clinical outcomes were collected prospectively. STEMI was defined according to the Universal Definition of Myocardial Infarction (24) as the presence of symptoms suggestive of myocardial ischemia, ECG changes compatible with acute MI (new persistent ST-

segment elevation and/or new or presumed new development of left bundle branch block at presentation), and elevation of cardiac biomarker (creatinine kinase (CK)-MB fraction at least twice the upper limit of normal or troponin I or T above individual hospital cut-off levels). MI size was estimated by peak levels of serum CK.

### **Study population**

We examined a cohort of consecutive patients with STEMI referred for PPCI within 12 hours of symptom onset to Swiss hospitals and included in the AMIS Plus registry from January 2008 to December 2014. Coronary angiography was performed via the radial or femoral artery. The use of antiplatelet agents and anticoagulation therapy were left to the discretion of the treating physician. PCI was defined as the use of any coronary device to approach, probe, or cross one or more coronary lesions, with the intention of performing a coronary intervention. The culprit lesion was identified and crossed with an angioplasty guidewire. Manual TA was performed at the discretion of the operator using one of the approved manual aspiration devices as per current practice in participating centers and followed by conventional PCI to the culprit vessel. No mechanical aspiration devices were included in this analysis.

Patients were divided into two study groups based on the use of manual thrombus aspiration before PCI (thrombus aspiration group) or PCI only (PCI-alone group). The thrombus aspiration group included all patients in whom manual thrombus aspiration was attempted. Patients were further divided into four time groups based on the time-of-day symptom onset, in line with recent literature (16, 17): group 1 (00:00-05:59), group 2 (06:00-11:59), group 3 (12:00-17:59) and group 4 (18:00-23:59).

### **Study outcomes**

Patient clinical, demographic and procedural characteristics, bleeding and procedural complications, all-cause mortality and final myocardial infarct size, determined by peak CK level, were obtained during admission. Epicardial reperfusion was assessed according to the Thrombolysis in Myocardial Infarction (TIMI) flow grading system before and after PCI (25). The primary outcome of the analysis was all-cause in-hospital mortality. The secondary outcome was myocardial infarct size.

### **Statistical analysis**

The results are presented as percentages for categorical variables and analyzed using the chi-square test or the Fisher's exact test as appropriate. Continuous normally distributed variables are expressed as means  $\pm$  standard deviation (SD) and compared using the two-tailed Student's t-test. Continuous non-



normally distributed variables are expressed as median and interquartile ranges and analyzed using the Mann–Whitney U test. A p-value of <0.05 was considered as statistically significant. According to previously published methodology (15), peak CK was plotted against hour at symptom onset expressed over a 24-hour interval and, in order to determine if the relation between myocardial infarction size and time of onset was circadian, a periodic sinusoidal regression analysis was performed using the equation  $f(t) = a + b \cdot \sin(2\pi \cdot (t-c)/24)$  where 'a' represents the rhythm mean value, 'b' indicates the amplitude of this sine function and 'c' indicates its origin. A 24-hour period for circadian rhythm was assumed and a circadian pattern was proved if the amplitude of the fitted curve was significantly different from 0 using the Wald test. Propensity-score matching analysis was performed to adjust for differences in demographic and procedural variables between the thrombus aspiration and the PCI-alone groups. Greedy matching in the form of nearest neighbor matching within a caliper of  $\pm 0.05$  on the propensity score was employed and randomly matched one patient in the thrombus aspiration group to one patient in the PCI-alone group. SPSS software (version 19.0.0, SPSS Inc., Chicago, Illinois, USA) was used for all statistical analyses.

### **Ethical considerations**

The study protocol complies with the Declaration of Helsinki regarding investigations on humans and was approved by the supra-regional ethics committee for clinical studies, the Swiss board for data security and regional ethics commissions.

### **RESULTS**

From 1<sup>st</sup> January 2008 to 31<sup>st</sup> December 2014, a total of 4'154 patients with STEMI from 51 Swiss hospitals were prospectively enrolled in the AMIS Plus registry; 3'648 STEMI patients underwent PPCI within 12 hours of symptom onset and were included for analysis. Of these, 1'800 patients (49%) underwent manual thrombectomy followed by PCI (thrombus aspiration group) and 1'848 patients (51%) were treated with PPCI only (PCI-alone group) (**Figure 1**).

### **Study population**

The baseline clinical, angiographic and procedural characteristics of the study population are listed in **Table 1**. Overall, patients in the thrombus aspiration group were significantly younger (60 vs. 64 years;  $p < 0.001$ ), more likely to be male (80% vs. 75.3%;  $p < 0.001$ ), and present with shorter reperfusion delays (symptom onset-to-primary PCI time: 185 vs. 195 minutes,  $p = 0.007$ ), compared with patients treated with PPCI only. Higher rates of hypertension (52.6% vs. 47.4%;  $p = 0.002$ ) and previous coronary artery bypass

grafting (2.9% vs. 1.9%;  $p=0.041$ ) were noted in the PCI-alone group. No significant differences were observed in the rates of cardiogenic shock and anterior MI between the treatment groups. Patients in the thrombus aspiration group were less likely to receive aspirin (5.9% vs. 8.0%;  $p=0.014$ ) or a P2Y<sub>12</sub> receptor antagonist (22.7% vs. 26.7%;  $p=0.008$ ) and present with complex coronary artery disease at admission, such as three-vessel (16.8% vs. 26.3%;  $p<0.001$ ), left main (1.1% vs. 2.6%;  $p=0.001$ ) and left anterior descending (43.8% vs. 48.7%;  $p=0.003$ ) coronary artery disease, compared with patients in the PCI-alone group. There were higher rates of glycoprotein IIb/IIIa inhibitors use in the thrombus aspiration group than in the PCI-alone group (40.3% vs 19.4%;  $p<0.001$ ). Procedural success rates, defined as rates of post-procedural TIMI flow grade 3, were significantly lower in thrombus aspiration group, as compared with patients who underwent PPCI only (93.8% vs. 94.3%;  $p=0.007$ ).

To account for confounding variables and bias between treatment groups, propensity-score matching was performed to adjust for differences in demographic and procedural variables producing a cohort of 2'860 patients (1'430 patients in the thrombus aspiration and PCI-alone groups) (**Figure 1**). Following propensity-score matching, baseline demographics and procedural characteristics were well balanced between the two propensity-matched cohorts (**Table 1**).

### **Circadian patterns of myocardial infarction incidence and myocardial infarct size**

A 24-hour variation in myocardial infarction onset time was observed in the overall STEMI population. The peak incidence of myocardial infarction symptom onset was observed during the 08:00-14:59 interval ( $n=1'300$ , 45.5%), whereas the lowest incidence occurred between 19:00 and 04:59 ( $N=857$ , 30.0%). When the incidence of STEMI was plotted against time-of-day symptom onset, the distribution fitted a sinusoidal function that fulfilled criteria for a significant circadian pattern ( $p<0.001$ ) (**Figure 2**). A significant circadian variation in final myocardial infarct size, as determined by peak CK release, was also demonstrated in the overall study population following PPCI. The distribution showed a sinusoidal function matching criteria for a significant circadian dependence between myocardial infarct size and time-of-day symptom onset ( $p<0.001$ ) (**Figure 2**). Myocardial injury was largest between 18:00 and 05:59, whereas smallest myocardial infarct size occurred during the 06:00-17:59 interval. Compared with patients in the PCI-alone group, those undergoing manual thrombectomy exhibited a significant relationship between time-of-day symptom onset and myocardial infarct size. The largest myocardial injury was observed in STEMI patients with symptom onset between 00:00 and 05:59 (group 1:  $2'723\pm 148$  U/l; group 2:  $2'493\pm 105$  U/l; group 3:  $2'550\pm 106$  U/l, and group 4:  $2'952\pm 144$  U/l;  $p=0.044$ ), while no significant differences were demonstrated in patients treated with PCI only (group 1:  $2'044\pm 124$  U/l; group 2:  $1'756\pm 93$  U/l; group 3:  $1'850\pm 128$  U/l,

and group 4:  $2'024\pm136$  U/l;  $p=0.239$ ).

### **In-hospital outcomes**

The incidence of all-cause in-hospital death was 4.3% in the thrombus aspiration group versus 3.4% in the PCI-alone group (hazard ratio 1.28; 95% confidence interval 0.87-1.89;  $p=0.20$ ) (**Table 2**). Patients undergoing manual thrombectomy versus PCI only had significant difference in final myocardial infarct size measured as peak CK release during hospital admission ( $2'636\pm61$  U/L vs.  $1'893\pm59$ ;  $p<0.001$ ).

A significant relationship between time-of-day symptom onset and myocardial infarct size was determined after nonlinear regression to a sinusoidal curve (**Figure 3**) in patients undergoing manual thrombectomy. Largest myocardial infarct size was observed in patients with symptom onset at 00:00. The amplitude of the sinusoidal curve (344 U/l) was significantly different from 0 ( $p<0.001$ ) and represented 14% of the mean myocardial infarct size. Importantly, there was significant greater benefit of manual thrombus aspiration with regards to myocardial salvage in patients with symptom onset between 06:00 and 17:59. Mean myocardial infarct size was 15% smaller in patients undergoing manual thrombectomy between 06:00 and 17:59, compared with patients undergoing manual thrombus aspiration in the 18:00-05:59 interval (mean difference for groups 2 and 3 versus groups 1 and 4: 391 U/l,  $p=0.01$ ). There was no significant circadian relationship between myocardial infarct size reduction and time-of-day symptom onset in patients treated with PCI only.

### **DISCUSSION**

The major findings of the present study from a large, prospective, nationwide cohort of unselected patients undergoing PPCI for STEMI can be summarized as follows: (1) routine manual TA during PPCI does not reduce in-hospital all-cause mortality when compared with PCI alone; (2) myocardial infarct size demonstrates a circadian dependence on the time of symptom onset with largest myocardial infarct size in patients with symptom onset between 22:00 and 02:00; and (3) effectiveness of manual TA during PPCI for STEMI varies according to the time of symptom onset, with largest myocardial salvage among patients with symptom onset between 06:00 and 17:59.

To the best of our knowledge, ours is the first study demonstrating the influence of circadian patterns on the clinical benefit of manual TA during PPCI for STEMI. These findings provide important clues to further understand the circadian pathophysiology of STEMI and might contribute to the identification of STEMI patients that may benefit from the selected use of manual TA during PPCI.

### **Circadian variation in acute myocardial infarction incidence and myocardial infarct size**

Consistent with the results of previous studies (26-29), findings from our large, nationwide cohort of consecutive STEMI patients referred for PPCI in contemporary clinical practice demonstrate a circadian variation of acute myocardial infarction onset time with a peak incidence in the early morning period at the sleep-to-wake transition confirming the critical influence of circadian rhythms on myocardial infarction pathophysiology. The circadian pattern of myocardial infarction incidence has been traditionally attributed to time-of-day-dependent fluctuations of noncardiac factors, such as increased sympathetic activity (30, 31), increased platelet aggregability (32, 33), decreased endogenous fibrinolytic activity (32, 34), and increased cortisol levels (35) during the early morning hours. Beyond the existence of circadian patterns of myocardial infarction incidence, we observed a circadian dependence of myocardial infarct size on the time of myocardial ischemia onset with largest myocardial injury occurring in patients with symptom onset between 22:00 and 02:00. These findings are in line with the results of numerous previous studies (14-19, 36) suggesting a circadian variation of myocardial infarct size in STEMI patients with largest myocardial infarct size occurring around midnight. Interestingly, the circadian pattern in myocardial infarct size persisted after adjustment for baseline characteristics and ischemic time, supporting the hypothesis of a circadian dependence of endogenous myocardial tolerance to I/R injury in humans.

### **Circadian variation in manual thrombus aspiration efficacy**

In our large, real-world nationwide registry of unselected STEMI patients undergoing PPCI, a strategy of routine manual thrombectomy as compared with PCI alone did not reduce the risk of in-hospital all-cause death. These findings are consistent with the results of two recent large-scale randomized clinical trials (6, 8). The multicenter, prospective, registry-based TASTE randomized trial (6) showed no significant benefit of routine manual thrombus aspiration with respect to all-cause mortality (2.8% vs. 3.0%, HR 0.94,  $p=0.63$ ), or any of other ischemic clinical outcomes at 30 days. Similarly, in the TOTAL trial (8), routine manual thrombus aspiration did not reduce rates of the primary composite outcome (cardiovascular death, recurrent myocardial infarction, cardiogenic shock, or NYHA class IV heart failure, 5.6% vs. 5.7%, HR 0.98,  $p=0.79$ ) and cardiovascular death (2.3% vs. 2.8%, HR 0.83,  $p=0.13$ ) within 30 days as compared to PCI alone. Finally, in a recent meta-analysis of seventeen randomized trials including 20'960 patients (10), aspiration thrombectomy was not associated with a significant reduction in the risk of all-cause mortality (2.8% vs. 3.2%, RR 0.89,  $p=0.13$ ) when compared with conventional PCI, irrespective of the follow-up duration (RR 0.86,  $p=0.36$  at 1 month; RR 0.82,  $p=0.17$  at 12 months).

The potential influence of time-of-day symptom onset and circadian rhythms on the clinical benefit of manual thrombus aspiration has not been reported previously. Based on data from our large nationwide registry of patients with STEMI, we demonstrate, for the first time to our knowledge a circadian relationship between the clinical benefit of manual thrombectomy and time of myocardial infarction onset. The greatest myocardial salvage was demonstrated in STEMI patients with symptom onset between 06:00 and 17:59, suggesting a potential benefit of manual thrombectomy when performed during the day time. The exact mechanism underlying the circadian dependence of manual thrombus aspiration efficacy on the time-of-day symptom onset cannot be directly derived from our study. However, our current knowledge concerning the role of human circadian rhythms in the pathogenesis of cardiovascular disease advocates for different possible explanations.

First, time-of-day-dependent variations in blood viscosity (37), coronary blood flow (38), plasma cortisol and catecholamine levels (39), platelet aggregation (32, 40) and activation (41), coagulation factors (32), and endogenous thrombolytic activity (42) have been previously reported. Platelet aggregation in response to epinephrine, adenosine diphosphate and thrombin is heightened during the early morning hours (32, 40). Similarly, plasma level of fibrinogen progressively increases between 06:00 and 12:00, whereas antithrombin levels decline (32). Time-of-day-dependent fluctuations in tissue-type plasminogen activator inhibition may contribute to both increased prothrombotic state during the early morning period, and enhanced natural fibrinolytic activity during the evening hours, regardless of behavioral influences and environment (43). These circadian patterns in platelet behavior and thrombotic propensity that result in 24-hour fluctuations of the physiological balance between prothrombotic and fibrinolytic factors may potentially explain time-of-day-dependent variations in the effectiveness of manual thrombectomy during PPCI for STEMI.

A 24-hour-dependent variation in the effectiveness of pharmacological and mechanical myocardial reperfusion strategies has been previously described in patients with STEMI. Thrombolytic therapy has been associated with lowest reperfusion rates in the early morning and late evening hours, independently of the type of thrombolytic agent, and highest efficacy when administered between noon and midnight (20, 21). In a study using multiple regression analysis, time-of-day of thrombolysis was found to be the independent factor with the greatest impact on successful reperfusion (44). This circadian pattern of resistance to thrombolysis has been explained by the circadian fluctuation of endogenous prothrombotic and fibrinolytic activities (20, 21). Both plasminogen activator inhibitor-1 (PAI-1) (20) and platelet surface activation markers such as glycoprotein Ib and P-selectin (41) have been shown to display a circadian pattern with highest PAI-1 levels and increased platelet aggregability in the early morning hours, which

coincides with the morning peak of thrombus formation and platelet aggregation (32, 34, 40). Similarly, a significant circadian variation with respect to microvascular perfusion, myocardial infarct size and clinical outcomes has been reported for STEMI patients undergoing PPCI (22, 45). After correction for baseline confounding factors, PPCI performed during the early morning hours between 04:00 and 08:00 was associated with increased 1-year mortality, whereas patients treated between 08:00 and 16:00 appeared to have improved myocardial perfusion and reduced long-term mortality rates. Failed myocardial reperfusion after PPCI in STEMI patients presenting during the early morning hours has been also associated with reduced 30-day mortality rates as compared to patients presenting between 06:00 and midnight (18). These findings tend to support the hypothesis of circadian variations in myocardial I/R tolerance in the human heart, which may potentially extend to myocardial salvaging interventions, such as manual intracoronary thrombectomy during PPCI.

Second, there is growing evidence from preclinical and observational studies suggesting a time-of-day-dependent variation in myocardial tolerance to I/R injury. Time-of-day-dependent variations in the cardiomyocyte circadian clock genes expression that modulate myocardial I/R tolerance throughout the 24-hour cycle have been previously observed in animal models. In a murine closed chest I/R model, hearts undergoing prolonged myocardial ischemia at the sleep-to-wake transition exhibited a 3.5-fold increase in myocardial infarct size after 24 hours of reperfusion, compared with those at the wake-to-sleep transition (46). Interestingly, the time-of-day-dependent variation in infarct size was abolished after deletion of the cardiomyocyte circadian clock gene (46). A similar circadian dependence of myocardial infarct size on the time of coronary artery occlusion has been described in humans. Patients with STEMI referred for PPCI experienced maximal myocardial injury when infarction started in the early morning hours and reperfusion occurred near to the sleep-to-wake transition, suggesting a comparable time-dependent mechanism of myocardial protection in the human heart (47). Furthermore, STEMI patients with failed myocardial reperfusion after PPCI presenting in the early morning hours have been shown to have greater tolerance to myocardial ischemia resulting in reduced 30-day mortality rates, compared with those presenting between 06:00 and midnight (18). These observations combined with our findings highlight a time-of-day-dependent variation in myocardial tolerance to I/R injury in the human heart, which may be relevant to clinical trials examining the effectiveness of interventions during PPCI for STEMI aimed at reducing myocardial infarct size, such as manual TA. Despite the precise mechanism remains unclear, a strong relationship between cascades mediating in I/R injury and those regulated by cardiomyocyte circadian clock genes has been recently suggested (13).

### **Study strengths and limitations**

The strength of the present study is that it includes a large, nationwide cohort of unselected patients with STEMI undergoing PPCI, thus reflecting the real-life management of STEMI patients in contemporary clinical practice and providing more appropriate event rates than traditionally reported in large randomized clinical trials including highly selected STEMI patients. Our study represents the first analysis evaluating the influence of circadian rhythms on the clinical efficacy of manual TA during PPCI for patients with STEMI, providing new insights into appropriate selection of patients that may derive benefit from intracoronary thrombectomy during PPCI. Our results highlight that time of symptom onset should be considered when designing future trials evaluating the potential clinical effect of intracoronary thrombectomy in STEMI patients treated with PPCI.

However, our data must be interpreted in view of several limitations. First, our study includes all limitations of a large nationwide registry, such as potential bias and unmeasured confounders associated with nonrandomized studies. In addition, we cannot exclude the possibility of underreporting of clinical outcomes. Despite the large size of our study population, our cohort may not have the power to detect differences in rates of in-hospital all-cause mortality related to the use of manual thrombectomy among subgroups of STEMI patients divided according the time-of-day symptom onset.

Second, the use of peak CK levels as a surrogate of myocardial infarct size to assess the circadian dependence of manual TA efficacy warrants comment. Myocardial infarct size was ascertained using peak CK measurements whereas recent non-invasive imaging modalities, such as cardiac magnetic resonance imaging (MRI) and single-photon emission computed tomography (SPECT), provide nowadays greater sensitivity and specificity than cardiac biomarkers for determination of myocardial infarct size. However, peak CK levels have been extensively used as a primary surrogate outcome to determine final myocardial infarct size in numerous previous studies evaluating circadian rhythms in patients with STEMI (14-19). Furthermore, previous studies have shown a strong correlation between peak CK levels and absolute myocardial infarct size, as determined by MRI and SPECT, in patients with STEMI undergoing PPCI (48, 49).

### **CONCLUSIONS**

In a large, real-world contemporary cohort of unselected patients undergoing PPCI for STEMI, routine manual thrombectomy did not reduce the risk of in-hospital all-cause death compared with PCI alone. However, we observed a circadian dependence of manual TA effectiveness with greatest myocardial salvage among patients with symptom onset occurring during the daytime. Our findings highlight the emerging concept of a circadian dependence of myocardial tolerance to I/R injury in the human heart and

suggest that adjustment of myocardial salvaging interventions such as manual intracoronary thrombectomy based on time-of-day symptom onset may be warranted for patients with STEMI treated with PPCI.



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## TABLE LEGENDS

### **TABLE 1: Baseline clinical, angiographic and procedural characteristics.**

Values are expressed as mean ( $\pm$  SD) or number (percentage). IQR: interquartile range.

- 5 MI: myocardial infarction; PCI: percutaneous coronary intervention; TIMI: Thrombolysis In Myocardial Infarction.

### **TABLE 2: In-hospital outcomes.**

SD: standard deviation.

## FIGURE LEGENDS

### FIGURE 1: Study flow chart.

PCI: percutaneous coronary intervention; STEMI: ST-segment elevation myocardial infarction.

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### FIGURE 2: Circadian variation of myocardial infarction incidence and myocardial infarct size.

Histograms represent the relationship between incidence of myocardial infarction (MI) (y-axis) and time-of-day symptom onset (x-axis) in the overall study population. Bar charts display number of patients with symptom onset within each of the daily time intervals, and demonstrate a lower incidence of MI during the night hours and a peak incidence of MI between 08:00 and 14:59 ( $p < 0.001$ ). Solid line represents the fitted sinusoidal curve of peak creatine kinase (CK) level (y-axis) plotted against time-of-day symptom onset (x-axis), demonstrating significantly largest myocardial infarct size in patients with symptom onset between 22:00 and 02:00 ( $p < 0.001$ ). Dashed lines represent 95% confidence interval.

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### 15 FIGURE 3: Circadian dependence of myocardial infarct size on time-of-day symptom onset in patients undergoing manual thrombectomy.

Solid line represents final myocardial infarct size, assessed by peak creatine kinase (CK) level (y-axis) plotted against time-of-day symptom onset (x-axis), demonstrating a sinusoidal curve that fulfills criteria for a significant circadian pattern ( $p < 0.001$ ). Dashed lines represent 95% confidence interval.